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1 Please give the title of the invention **NOVEL PROTEIN KINASE RECEPTORS**

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NOVEL PROTEIN KINASE RECEPTORS

of the Invention

The present invention relates generally to novel human protein kinase receptors and to the nucleic acid molecules encoding them. More specifically, the invention relates to the isolation of DNA sequences encoding kinase receptors with homology to the transforming growth factor β type II family of receptors.

Background to the Invention

The transforming growth factor-beta (TGF-beta) superfamily consists of a family of structurally related proteins, including TGF-betas, activins, inhibins, Mullerian inhibiting substance and bone morphogenic proteins (BMPs), which regulate cell proliferation, differentiation and various other cell functions (for review see Roberts and Sporn, (1990), Peptide Growth Factors and their Receptors, part I, Sporn and Roberts eds Springer-Verlag, Berlin, pp 419-472; Massague (1990), Annu. Rev. Cell Biol. 5, 597-641; Vale et al (1990), Peptide Growth Factors and their Receptors, part I, Sporn and Roberts eds, Springer Verlag, Berlin, pp 419-472; Cate et al, (1990), Peptide Growth Factors and their Receptors, part I, Springer Verlag, Berlin pp 179-210). The action of these growth factors is mediated through binding to specific cell surface receptors. Within this family, TGF-beta receptors have been most thoroughly characterized. By covalently crosslinking radiolabeled TGF-beta to cell surface molecules followed by polyacrylamide gel electrophoresis of the affinity labeled complexes, three distinct size classes of cell surface proteins (in most cases) have been identified, named receptor type I, type II and type III (or betaglycan), (For review see Massague, (1992), Cell 69, 1067-1070). Current evidence suggests that type I and type II receptors are involved in receptor signal transduction (Segarini, (1989) et al, Mol. Endo., 3 261 - 272 Laiho et al (1991), J Biol. Chem. 266 9100-9112), and that the type III receptor (betaglycan) has a more indirect role, possibly by facilitating the binding of ligand to type II receptors (Lin et al (1992) Cell 68 775-785).

Binding analysis with activin A and BMP4 have led to the identification of two coexisting crosslinked affinity complexes of 50-60kDa and 70-80 kDa on responsive cells (Hino et al,

(1989). J Biol. Chem. 264, 10309-10314; Mathews and Vale (1991), Cell 68 775-785; Paralkar et al (1991), Proc. Natl. Acad. Sci. USA, 87 8913-8917). In analogy with TGF-beta receptors they are thought to be signalling receptors and have been named rec type I and type II.

Molecular cloning and sequence determination of the mouse activin receptor II (mActR-II) cDNA (Mathews and Vale, (1991) Cell 65, 973-982) revealed the predicted structure of the receptor to be a transmembrane protein with an intracellular serine/threonine kinase domain. The activin receptor is related to the C. elegans daf-1 gene product, the ligand is currently unknown. (Georgi, et al. (1990), Cell 61, 635-645). Recently, ActR-II was shown to be a serine/threonine/tyrosine protein kinase (Nakamura et al (1992), J Biol Chem 267, 18924-18928). Another subtype of the activin receptor, Act-R-IIB of which there are different splicing variants, has recently been cloned, and also appears to code for a receptor-type serine/threonine kinase. (Matthews et al (1992), Science 255, 1702-1705; Attisano et al (1992), Cell 68, 97-108). The TGF-beta type II receptor cDNA was also isolated recently, using an expression cloning strategy, and shown to be a functional transmembrane serine/threonine kinase receptor (Lin et al (1992), Cell 68, 775-785). Although the kinase domains show significant homology, the extracellular domains of ActR-II, Act-R-IIB and TGF-beta type II show little sequence similarity to each other, although they all contain a cysteine rich region.

To ascertain whether there were other members of this family of receptors, a protocol was designed to clone ActR-II/daf 1 related genes. This strategy made use of the polymerase chain reaction (PCR), using degenerate primers based upon the amino acid sequence similarity between the kinase domains of mAct R.II and daf 1 gene products. This strategy resulted in the isolation of a new family of receptor kinases. These genes showed a 13-39% sequence similarity with ActRII and TGF-beta RII and 40-92% sequence similarity towards each other in the kinase domains.

These cloned genes can be used to express the receptors, which can then be used to isolate the respective ligands. Agonists and antagonists may be designed which may be useful in regulating receptor functions. Antibodies may also be raised against the receptors and used in immunoassays and related analytical methods, to measure and detect the receptors as well as modulate their activity.

BRIEF DESCRIPTION OF THE FIGURES

- Figure 1** Alignment of the serine/threonine (S/T) kinase domains of related receptors.
- Figure 2** Sequence and characteristics of the primers used in the initial PCR reactions.
- Figure 3** Northern blot analysis of various cell lines - A549 (lanes 1 & 2), HEL without (lane 3) and with TPA treatment (lane 4), HSC-2 (lane 6), MCF7 (lane 7), T47D (lanes 8 & 9), LNCAP (lanes 10 & 11). Cells were cultured without serum for lanes 1, 6, 8 and 10. Hybridised with PCR product 11.1 or 11.2.
- Figure 4** Northern blot analysis of various cell lines. HEL and TPA (lane 1), HEL (lane 2), AG1518 (lane 3), PC3U (lane 4). Hybridised with labelled PCR product 11.3.
- Figure 5** Nucleotide sequence and deduced amino acid sequence of ARK-1 (clone HP57).
- Figure 6** Nucleotide sequence and deduced amino acid sequence of ARK1 (clone 3F).
- Figure 7** Nucleotide sequence and deduced amino acid sequence of ARK-2.
- Figure 8** Nucleotide sequence and deduced amino acid sequence of ARK-3.
- Figure 9** Partial nucleotide sequence and deduced amino acid sequence of ARK-4.
- Figure 10** Partial nucleotide sequence and deduced amino acid sequence of ARK-5.

- Figure 13** Partial nucleotide sequence and deduced amino acid sequence of PCR product 3.1.
- Figure 14** Partial nucleotide sequence and deduced amino acid sequence of PCR product 1/2-6.
- Figure 15** Partial nucleotide sequence and deduced amino acid sequence of PCR product 1/2-7.
- Figure 16** Schematic diagram of the ARK-1 gene and protein.
- Figure 17** Schematic diagram of the ARK-2 gene and protein.
- Figure 18** Schematic diagram of the ARK-3 gene and protein.
- Figure 19** Schematic diagram of ARK-4 and ARK-5 gene and protein.
- Figure 20** Sequence alignment between the ARKs.
- Figure 21** Sequence alignment of the cysteine rich domains of the ARKs, TGF β RII, Act-R-II, Act-R-IIB and daf-1 receptors.
- Figure 22** Northern blot analysis of various human tissues hybridised with EMBLA, lane 1 - heart, lane 2 - brain, lane 3 - placenta, lane 4 -lung, lane 5 - liver, lane 6 - skeletal muscle, lane 7 - kidney, lane 8 - pancreas.

EXAMPLE 1

To examine the possibility that other receptors for the TGF-beta superfamily possessed sequence similarity, a study was initiated aimed at the cloning of any ActR-II/daf-1 related genes. A polymerase chain reaction (PCR) strategy was designed using degenerate primers based upon the amino acid sequence similarity between the kinase domains of the mActR-II and daf-1 gene product. Figure 1 shows the aligned serine/threonine kinase domains (I-VIII), using the nomenclature of the subdomains according to Hanks et al, (1988), Science 241 42-52 of four related receptors of the TGF-beta superfamily - hTGFBR-II, ActR-IIB, mActR-II and the daf-1 gene product.

Several considerations were applied in the design of the PCR primers. The sequences were taken from regions of homology between the activin receptor II and the daf-1 gene product, with particular emphasis on residues that confer serine/threonine specificity and on residues that are shared by transmembrane kinase proteins and not by cytoplasmic kinases. The primers were designed so that each primer of a PCR set had an approximately similar GC composition, and so that self complementarity and complementarity between the 3' ends of the primer sets were avoided. Degeneracy of the primers was kept as low as possible, in particular avoiding serine, leucine and arginine residues (6 possible codons) and human codon preference was applied. Degeneracy was particularly avoided at the 3' end as unlike the 5' end, where mismatches are tolerated, mismatches at the 3' end dramatically reduce the efficiency of PCR.

In order to facilitate directional subcloning, restriction enzyme sites were included at the 5' end of the primers, with a GC clamp, which permits efficient restrictive enzyme digestion. The primers utilised are shown in Figure 2. The various oligonucleotides were synthesised on an oligonucleotide synthesizer according to the manufacturer's instructions. For the initial PCR reactions, mRNA was isolated from a human erythroleukaemia cell line (HEL 92.1.7) obtained from the America Type Culture Collection (ATCC TIB 180). These cells were chosen as they have been shown to respond to both activin and TGF-beta. Moreover leukaemic cells have proved to be rich sources for the cloning of novel receptor

tyrosine kinases (Partanen *et al* (1990) Proc. Natl. Acad. Sci. USA 87 8913-8917 and '992) Mol Cell Biol. 12, 1698-1707). RNA was prepared using the guanidinium isothiocyanate method (Chirgwin, *et al*, (1979) Biochemistry 18 5294-5299). mRNA was selected using the poly AT tract system (Promega Madison [WI]) as described by the manufacturer's instructions.

The PCR reactions were carried out as follows:- 2µg of mRNA was reverse transcribed in the presence of 50mM Tris-HCl, pH 8.3, 8mM MgCl₂, 30mM KCl, 10mM DTT, 2mM NTPs, excess oligo dT primers and 34 units of AMV reverse transcriptase at 42°C for 2 hours. PCR was performed with a 7.5% aliquot of the reverse transcribed mRNA, in the presence of 50mM KCl, 10mM Tris-HCl pH 8.3, 1.5mM MgCl₂, 0.01% gelatin, 0.2mM NTPs, 1µM of both sense and antisense primers, using 2 units of Taq polymerase. The PCR programme was 5 cycles for 1 minute at 94°C, followed by 1 minute at 50°C, followed by a 2 minute ramp to 72°C, then 1 minute at 72°C. This was followed by 20 cycles of 1 minute at 94°C, followed by 30 seconds at 55°C, followed by 1 minute at 72°C. From the reaction mix a 0.3% aliquant of the material was taken and processed for a second round of PCR amplification. This involved 25 cycles of 1 minute at 94°C, followed by 30 seconds at 55°C followed by 1 minute at 72°C.

General nucleic procedures such as purification of nucleic acids, restriction enzyme digestion, gel electrophoresis, transfer of nucleic acid to solid supports and subcloning were performed essentially according to established procedures as described by Sambrook, Fritsch and Maniatis (1989) Molecular Cloning; a Laboratory Manual. Cold Spring Harbour Laboratory (Cold Spring Harbour, New York, USA).

A sample of the PCR products was analyzed by standard agarose gel electrophoresis for size. Those with a similar size to the relevant sections of the activin receptor II and daf-1 genes (see Table 1), were digested with the relevant restrictive enzymes (BamHI and EcoRI) and subcloned into the pUC plasmid, which had been previously linearised with BamHI and EcoRI. The recombinants were transformed into E. coli. and plated. Individual clones were sequenced using standard double stranded sequencing techniques using the dideoxynucleotide chain termination method as described by Sanger *et al*; Proc. Nat. Acad. Sci USA 74, 5463-5467, using T7 DNA polymerase.

Sequence identity is defined as the number of residues that are identical between 2 sequences. Sequence similarity comparisons were performed using the clustal V multiple sequence alignment programme (DNA) star. (Higgins and Sharp (1989) CABIOS 5, 151-153.

TABLE 1

NAME	PRIMERS	INSERT SIZE (In bp)	SIZE OF DNA FRAGMENT IN mActR/hTGFB IN CLONES (In bp)	SEQUENCE IDENTITY WITH SEQUENCE mActRII/hTGFBRII (In %)	SEQUENCE IDENTITY BETWEEN mActRII AND TGFBRII (In %)
11.1	B3-S/E8-AS	460	460	46/40	42
11.2	B3-S/E8-AS	460	460	49/44	47
11.3	B3-S/E8-AS	460	460	44/38	48
11.29	B3-S/E8-AS	460	460	ND/100	ND
9.2	B1-S/E8-AS	800	795	100/ND	ND
5.1	B7-S/E8-AS	140	143	33/33	60
5.2	B7-S/E8-AS	140	143	40/38	60
3.1	B7-S/E8-AS	90	143	58/67	79
1/2.7	B7-S/E8-AS	200	143	38/35	60
1/2.7	B7-S/E8-AS	200	143	43/37	60

EXAMPLE 2

Northern blots were performed in order to analyse the expression of the different genes to look for interesting expression patterns and also to identify an appropriate source from which to attempt to clone the full length cDNAs.

RNA was prepared from the following cell lines: HEL 92.1.7 (Human Erythroleukaemia), PC-3 (human prostate carcinoma), A549 (human lung adenocarcinoma), T47D and MCF-7 (human breast carcinomas) obtained from the American Type Culture Collection (Rockville MD). PC-3U4 (human prostate carcinoma) obtained from Sten Nilsson at the Department of Oncology, University Hospital, Uppsala, Sweden. HSC-2 (human squamous carcinoma) obtained and isolated as described by Momose et al (1989), J Oral Pathology. Med 18

391-395. AG 1518 (Human foreskin fibroblasts) obtained from the Human Mutant Cell Repository, Camden New Jersey. LNCAP (Human prostate carcinoma) obtained from the Human Tumour Cell Line Repository, Rye, New York, USA.

10 μ g of RNA or 2 μ g of mRNA samples were fractionated on agarose gels in the presence of formaldehyde and then transferred onto strengthened nitrocellulose or nylon membranes. The PCR products 11.1, 11.2 and 11.3 were radiolabelled using the random priming method (Feinberg and Vogelstein (1983), Anal Biochemistry 132 6-13) using a multi-prime DNA labelling system and [α - 32 P]dCTP. The filters were hybridised with the probes at 42°C over night in 50% formaldehyde, 5 x SSC, 0.1% SDS, 50 mM sodium phosphate and 0.1mg/ml salmon sperm DNA. The filters were washed with 0.5 x SSC, 0.1% SDS at 55°C before being exposed to X-ray film.

The results are shown in figures 3 and 4. Genes hybridizing with PCR products 11.1 and 11.2 appear to be ubiquitously expressed, whereas the gene hybridizing with PCR product 11.3 is expressed in fibroblasts (figure 4) whereas expression was not detected in A549, HEL, HSC-2, MCF-7, T47D and LNCAP cells (data not shown).

EXAMPLE 3

In order to obtain full length clones for the various PCR products, the PCR probes were used to screen various cDNA libraries. The clones were isolated from the libraries using standard molecular biological techniques. The human placental lambda ZAPII DNA library was obtained from Hideo Toyoshima, University of Tokyo, Japan. (cDNA clones HP22, HP57, HP29, HP53 and HP83 were obtained from this library). The human fibroblast AG1518 lambda ZAPII, cDNA and HEL cell lambda gt10 cDNA libraries were prepared with

Ribo clone cDNA synthesis system (Promega) and Giga pack Gold (Stratagene) and lambda gt10 in vitro packaging kit (Amersham) respectively, according to the manufacturer procedures, cDNA clones ONF1, ONF2, ONF3, ONF4 and ONF5 were obtained from ... human fibroblast library. The cDNA clone EMBLA was obtained from the HEL lambda lgt10 cDNA library. The human fibroblast AG1518 lambda gt10 library was obtained from Lena Cleasson-Welsh as described in Cleasson-Welsh et al. (1989), Proc. Nat. Acad. Sci. USA 86 4917-4921. The cDNA clones ON and 3F were obtained from this library. The HEL cell lambda gt11 cDNA library was obtained from Mortimer Poncz and was described in Poncz et al. (1987) Blood 69 219-223. The cDNA clone 11H8 was obtained from this library.

The double stranded DNA clones were all sequenced using the dideoxynucleotide chain-termination method as described by Sanger et al. (1977), Proc. Nat. Acad. of Sci. USA 74 5463 - 5467, using T7 DNA polymerase.

Analysis of the sequences obtained revealed the existence of five distinct putative receptor serine/threonine kinases which have been named ARK1-5 (Activin-receptor Related Kinases). The relation of the cDNA clones to each other is shown below.

To summarise clones 3F, HP22, HP57, ONF1, ONF3, ONF4 and HP29 encode the same gene ARK-1. HP53, HP64 and HP84 encode the same gene ARK-2. ONF5, ONF2 and ON11 encode the same gene ARK3. 11H8 encodes a different gene ARK-4. EMBLA encodes ARK-5.

Two of the clones which encode the ARK-1 gene, HP57 and 3F have been sequenced. These are shown in figures 5 and 6, respectively. These were both obtained by screening

The nucleotide sequence and deduced amino acid sequence of the activin receptor related kinase-5 (ARK-5) is shown in figure 10. This is the sequence of the cDNA clone λ JLA which was obtained by screening the HEL cDNA library with PCR products 11.1 and 11.2 as a probe.

The sequence similarity between the five ARK genes and TGF β RII, ActR-IIB and mActR-II in the serine/threonine kinase domains is shown in figure 11. The five members of this family have a 40-49% sequence similarity in the serine/threonine kinase domain with approximately 35% sequence similarity to the same domain in the Activin and TGF-beta RII receptors. The increased homology between the ARK receptors as compared to the TGF β RII and the activin receptors places them in a distinct family.

Further novel sequences have been obtained from other PCR products. Figures 12-15 show the nucleotide sequences and deduced amino acid sequences of a series of PCR products arising from a reaction using human erythroleukaemia cells as the mRNA source in the cDNA preparation and primers B7-S and E8-AS as described supra. The primer sequences are included and these sequences, in particular those forming the restriction sites, may not correspond to the actual gene sequence from which the PCR product was derived. These partial cDNA sequences can be used to isolate the full length sequences from the cDNA libraries described above.

Sequence analysis of the cDNAs for this family of genes revealed that they code for proteins with a ligand binding extracellular domain with a characteristic cysteine rich region, a single hydrophobic membrane spanning domain and an intracellular kinase domain with a predicted serine/threonine specificity. The recent finding that the of ActR-II receptor has serine/threonine/tyrosine kinase activity (Nakamura *et al* (1992), J Biol. Chem.

267, 18924-18928), suggest that these kinases may also have tyrosine kinase activity.

Schematic diagrams of each of the five genes described, are shown in figures 16-19. A sequence alignment between the genes is shown in figure 20. Alignment between the cysteine rich domains of the ARK cDNAs is shown in figure 21. The homology is low, approximately 10-20%, however, the positions of the cysteine residues are conserved, suggesting that the receptors probably bind different but related ligands.

Further Northern analysis was performed to study the expression of ARK-5 in various human tissues. Figure 21 shows that ARK-5 appears to be ubiquitously expressed as a 5.5kb message in various tissues. Expression is particularly high in the brain and skeletal muscle.

The foregoing describes the isolation of nucleic acid sequences coding for a new family of human receptor kinases. The term nucleic acid molecules as used herein refers to any sequence which code for the human form, amino acid sequences of which are presented herein. It is understood that the well known phenomenon of codon degeneracy provides for a great deal of sequence variation and all such varieties are included within the scope of this invention. The nucleic acid sequence described in the application may be used to clone the respective genomic DNA sequences in order to study the genes' structure and regulation. The human cDNA or genomic sequences can also be used to isolate the homologous genes from other mammalian species. The mammalian DNA sequences can be used to study the receptors' functions in various in vitro and in vivo model systems.

It is also to be recognized that given the sequence information provided herein, the artisan could easily combine the molecules with a pertinent promoter in a vector, so as to produce

a cloning vehicle. The promoter and coding molecule must be operably linked via of the well recognized and easily practised methodologies for so doing. The resulting constructs, as well as the isolated nucleic acid molecules themselves, may be used to transfect cells, such as the prokaryotic cells (eg, E. coli), or eukaryotes such as yeast (S. cerevisiae), COS or CHO cell lines. Other recipient cells will also be apparent to the skilled artisan.

Several methods may be used to isolate the ligands for the ARKs. cDNA clones encoding the active open reading frames can be subcloned into expression vectors and transfected into eukaryotic cells, for example COS cells. The transfected cells which can express the receptor will be subjected to binding assays for radioactively labelled members of the TGF-beta superfamily (TGF-beta, activins, inhibins, bone morphogenic proteins and mullerian inhibiting substance), as it might be predicted that the receptors will bind members of the TGF-beta superfamily. Various biochemical or cell based assays can be designed to identify the ligands, in tissue extracts or conditioned media, for receptors for which a ligand is not found. Alternatively, purified receptor could be used to isolate the ligands using an affinity based approach. The determination of the expression patterns of the receptors may also aid in the isolation of the ligand. These studies may be carried out using the ARK DNA or RNA sequences to perform in situ hybridisation studies. Antibodies raised to the receptors may also be used to perform this analysis.

The use of various model systems or structural studies should enable the development of specific agonists and antagonists useful in regulating receptor function. These molecules would either mimic the effect of the respective ligand (agonists) or block the effect of the ligand (antagonists). It may be envisaged that these can be amino acid containing molecules, antibodies or other molecules able to interact with the receptors.

The foregoing constitutes examples of the invention applicants intend to claim which includes inter alia, isolated nucleic acid molecules coding for Activin receptor Related kinases (ARKs), as defined herein. These include such sequences isolated from mammalia species such as human, mouse, rat, rabbit and monkey.

It will be understood that the specification and examples are illustrative but not limitative of the present invention and that other embodiments within the spirit and scope of the invention will suggest themselves to those skilled in the art.

③

Figure 1

S/T kinase domains (I to VIII) from transmembrane proteins

cons.aa	<u>GGGV</u>	<u>AK</u>	<u>E</u>
hTGFBR-II	LDTLVGKGRFAEVYKAKLKQNTSEQFETVAVKIFPYDHYASWIKDKDIFSDINLKHENILQF		
mActR-IIB	LLEIKARGRFGCVWKAQLMN-----DFVAVKIKPLQDKQSWQSEREIFSTPGMKHENLLQF		
mActR-II	LLEVKARGRFGCVWKAQLLN-----EYVAVKIFPIQDKQSWQNEYEVYSIPGMTHENILQF		
daf-1	LTGRVGSGRPGNVSRGDYRG-----EAVAVKVFNAIDEPAFHKEISIFETRLRHPNVRLY		
subdomains	I	II	III IV

hTGFBR-II	LTAEERKTELGKQYWLITAFHAKGNLQEYLTRHVISWEDLRNVGSSSLARGLSHLHSDHTP-C
mActR-IIB	IAAEKRGSNLEVELWLITAFHDKGSLIDYLKGNII TWNELCHVAETMSRGISY LHEDVPWCR
mActR-II	IGA EKRGTSVDVDLWLITAFHEKGSLSDFLKANVVSWNELCHIAETMARGLAYLHEDI PGLK
daf-1	IGSDRVDTGFTLWLVI EYHPSGSLHDFLLENTVNIETYYNLARSTASGLAFLHNQIGGSK
subdomains	V VI-A

cons.aa	<u>DLK N</u>	<u>DFG</u>
hTGFBR-II	-GRPKTPIVHRDLKSSNII LVKNDLTCCLCDFGLSLRL---GPYSSVDDLANSQGQVGTARYMAP	
mActR-IIB	GECHKPSIAHRDFKSKNVLLKSDLTAVLADFGFLAVRF---EPGKPPGD--THGQVGTRRYMAP	
mActR-II	-DGHKPAISHRDIKSKNVLLKNNLTACIADFGFLALKF---EAGKSAGD--THGQVGTRRYMAP	
daf-1	-ESNKPAMAHARDIKSKNIMYKNDLTCAIGDLGLSLSKPEDAASDI IAN--ENYKCGTVRYLAP	
subdomains	VI-B VII VIII	

(nomenclature of the subdomains according to Hanks et al. 1988)

Fig. 2

<u>Name</u>	<u>Sequence and characteristics</u>
-------------	-------------------------------------

BI-S sense primer, extracellular domain, cysteine rich region,
BamHI site at 5' end, 28 mer, 64 fold degeneracy

a.a C C E G N M C
5' GCGGATCCTGTTGTGAAGGNAATATGTG 3'
BAMHI C C G C

BZ-S sense primer, kinase domain I
BamHI site at 5' end, 25 mer, 384 fold degeneracy

a.a G R F G C V
5' GCGGATCCGGNCGCTTTGCGTGTGT 3'
BamHI A A C C
G

B3-S sense primer, kinase domain II
BamHI site at 5' end , 25 mer, 162 fold degeneracy

a.a V A V K I F
5' GCGGATCCGTCGCAGTCAAAATTTT 3'
BamHI G C G G C
 T T T A

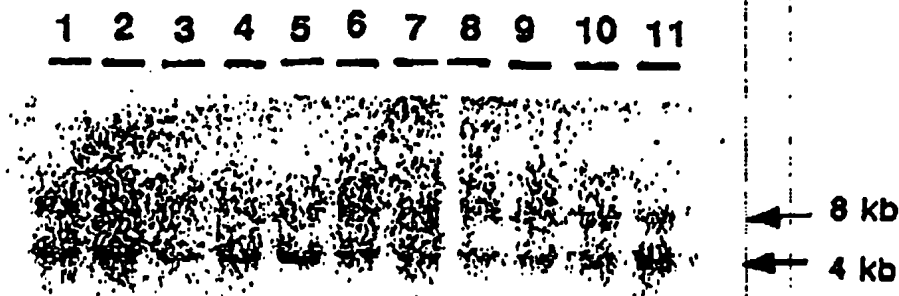
B7-S sense primer, kinase domain VIB, S/T kinase specific residues
BamHI site at 5' end, 24 mer, 288 fold degeneracy

a.a R D I K S K N
5' GCGGATCCGCGATATTAAAGCAA 3'
 BAMHI A C C GTCT
 G A

E8-AS anti sense primer, kinase domain, S/T kinase specific residues
EcoRI site at 5' end, 20 mer, 18 fold degeneracy

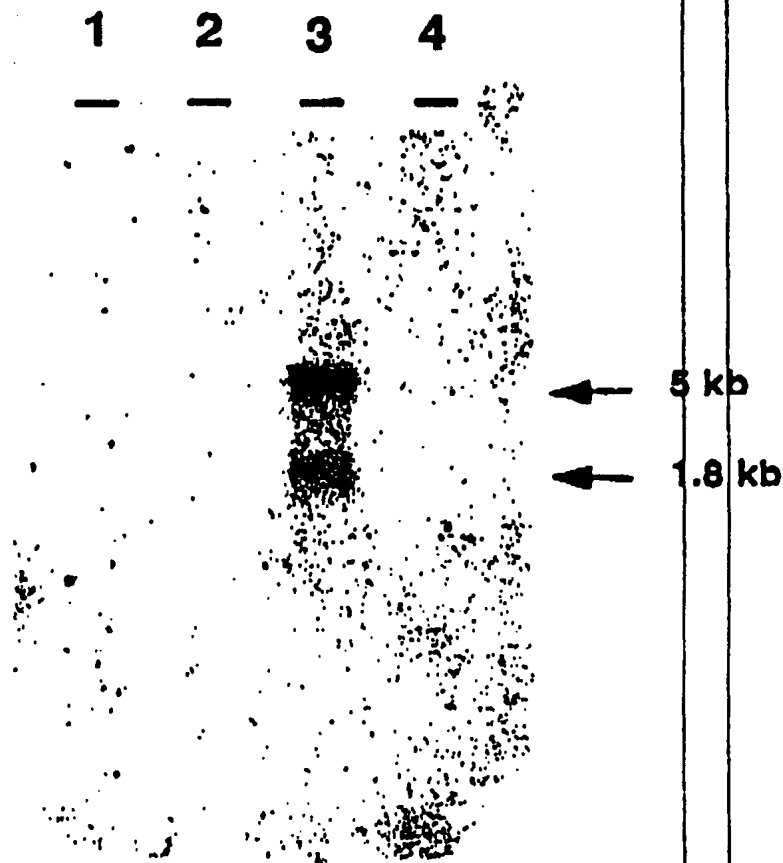
a.a E P A M Y
5' CGGAATTCTGGTGCCATATA
 E C O R I G G G
 A A

Figure 3

**Northern blot analysis**

Total RNA (10 microgram) from A549 (lanes 1 and 2), HEL without (lane 3) and HEL with TPA treatment, 1.6 microM for 24 hr's (lane 4), HSC-2, MCF-7 (lanes 6 and 7), T47D (lanes 8 and 9), LNCAP without (lane 10) and with treatment of synthetic androgen R1881 (300 microgram/ml) for 48 hr's., lane 11). RNA from cells in lanes 2, 6, and 9 were cultured without serum addition to the media. RNA from cells in lanes 1, 3, 4, 5, 7, 8, 10 and 11 were cultured in the presence of serum. Upon fractionation the RNA was transferred to Hybond N and hybridized with radiolabeled PCR product 11.1 or 11.2 at 42 C in the presence of 50 % formamide and 5 x SSC (both probes resulted in identical pattern). Filter was washed with 0.5 x SSC, 0.1 % SDS at 55 C.

Figure 4.

**Northern blot analysis**

RNA from HEL treated with TPA (lane 1; 2 microgram mRNA), HEL (lane 2; 2 microgram mRNA), AG1518 (lane 3; 2 microgram mRNA) and PC3U (lane 4; 10 microgram total RNA) was transferred to Hybond C-extra and hybridized with radiolabeled PCR product 11.3 at 42 C in the presence of 50 % formamide and 5 x SSC. Filter was washed with 0.5 x SSC, 0.1 % SDS at 55 C.

ARK-1

HP 57

Figure 5

(2)

CAATTCGGCGCCGCGGATCCAAGGAAACGGTTTATTAGGAGGGAGTGGTGGAGCTGGGCCAGGCAGGAA 70
GACGCTGGAATAAGAAACATTTTTTCTCCAGCCCCATCCCAATCCCGGAGGCTGCCGCCAGGCTCCG 140
CCGAGCGAGCCCTCCCCGGCTCCAGCCCGTCCGGGGCCCGCCGGAGCCCGAGCCCGGCTCCAGCGCT 210
GGCGGTGCAACTCCGGCCGCGCGGTGGAGGGGAGGTGGCCCCGGTCCGCCAAGCTAGCGCCCCGCCACC 280
CGCAGACCCGGCCAGAGGGACCATGACCTTGCGCTCCCCAGGAAAGCCCTTCTGATGCTGCTGATGGC 350
H T L G S P R K Q L L H L L H A
CTTGGTGACCCAGCGAGACCCTGTGAAGCCGTCTCGCGCCCGCTGGTGACCTCCACGTGTGAGAGCCCA 420
L Y T G G D P V K P S R G P L V T C T C E S P
CATTCGAAGGGGCTACCTGCCGGGGGGCTGGTGACAGTAGTGTGGTGGGGAGGAGGGGAGGCACC 490
H C K G P T C R G A V C T V V L V R E E R R H
CCCAAGAACATCGGGGCTCGGGGAACCTGCACAGGAGCTCTGCAAGGGGCCCGCCACCGACTTCGTCAA 560
P Q E H R G C G N L H R E L C R G R P T E F V N
CCACTACTGCTCGGACAGCCACCTCTGCAACCACAACGTGTCCCTGGTGGTGGAGGCCAGCCAACCTCT 630
H Y C C D S H L C N H N Y S L V L E A T Q P P
TCGGAGCAGCCGGGAACAGATGGCCAGCTGGCCCTGATCCTGGGCCCCGTGCTGGCCTTGTGGCCCTG 700
S E O P G T D G O L A L I L G P V L A L L A L
TGGCCCTGGGTGTCTGGGCTGTGGCATGTCCGACGGAGGCAGGAGAAACAGCGTGCCCTGCACAGCGA 770
V A L G V L G L W H Y R R R O E K O R G L H S E
GCTGGGAGAGTCCAGTCTCATCTGAAAGCATCTGAGCAGGGGACACAGATGTTGGGGACCTCTGQAC 840
L G E S S L I L K A S E G G D T H L Q D L L D
AGTCACTGCACCACAGGAGTGGCTCAGGGCTCCCTTCCCTGGTGCAGAGGACAGTGGCAGCGCAGGTTG 910
S D C T T G S O S G L P F L V O R T V A R Q V
CCTTGGTGGAGTGTGTGGGAAAAGGCCCTATGCCGAAGTGTGGCGGGGCTTGTGGCACGGTGAGAGTGT 980
A L V E C V G K G R Y G E Y W R G L W H G E S Y
GGCGTCAAGATCTTCTCCTCGAGGGATCAACATCTCTGTTCCGGGAGACTGAGATCTATAACACAGTA 1050
A V K I F S S R D E O S W F R E T E I Y N T Y
TTGCTCAGACAGGACAACATCCTAAGCTTCATCGCTCAGACATGACCTCCCGCAACTCGAGCAGGCAGC 1120
L L R H D N I L G F I A S D H T S R H S S T G
TGTGGCTCATCAGGCACTACGACGAGCAGGCTCCCTCTACGACTTCTGCAGAGACAGAGCTGGAGCC 1190
L V L I T H Y H E H G S L Y D F L O R O T L E P
CCATCTGGCTCTGAGGCTAAGTGTGTCCGGGGCATCGGGCTGGCCACCTGCACGTGGAGATCTTCGGT 1260
H L A L R L A V S A A C G L A H L H V E I F G
ACACAGGCAAAACCAACCATGCCCCACGGGACTTCAAGAGCCCAATGTGCTGGTCAAGAGCAACCTGC 1330
T G G K P A I A H R D F K S R N V L V K S N L
AGTGTTCATCGCGGACCTGGGCTGGCTGTGATGCACTCAGCGGCAGCGATTACCTGGACATCGGCAA 1400
O C C I A D L G L A V N H S O G S D Y L D I G N
CAACCCGAGAGTCCCGACCAAGCGGTACATGGCAGCCGAGGTGCTGGAGGAGCAGATCCCGACGGACTGC 1470
H P R V O T K R Y M A P E V L D E Q I R T D C
TTTGAATCTACAAGTGGACTGACATCTGGGCTTTGGCCTGGTGTGTGGGAGATTGCCCCGCGACCA 1540
F E S Y K W T D I V A F G L V L W E I A R R T

HP 57

(3)

TCGTGAATGGCAGTCGTGGAGGACTATAGACCACCCCTCTATGATGTGGTGGCCCAATGACCCAGCTTTGA 1610
I V N G I V E D Y R P P F Y D V V P N D P S F E
GGACATGAAGAAGGTGGTGTGTGTGGATCAGCAGACCCCAACCATCCCTAACCGGCTGGCTGCAGACCCG 1680
D H K K V V C V D Q Q T P T I P N R L A A D P
GTCTCTCAAGCCTAGCTCAGATGATGCGGGAGTGCTGGTACCCAAACCCCTCTGCCCCGACTCAGCGCGC 1750
Y L S G L A Q M H R E C V Y P N P S A R L T A
TGCGGATCAAGAAGACACTACAAAAAATTAGCAACAQTCCAGAGAAGCCTAAAGTGATTCAATAGCCGAG 1820
L R K K T L Q K I S N S P E K P K V I Q
GAGCACCTGATTCTTTCTGCTGCAAGGGGGCTGGGGGGGTGGGGGGCAQTGGATGCTGCCCTATCTGGG 1890
TAGAGGTAGTGTGAGTGTGGTGTGTGCTGGGGATGGGCAGCTGCCTGCTGCTCGCCCCCAGCCAC 1960
CCAAGCAAAATACAGCTGGGCTGAAACCTGAAAAAAAAAAAAA 2008

Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 1 (ARK-1). The complete sequence of cDNA clone HP57 is shown, which was obtained by screening a human placenta cDNA library with PCR product 11.3 as a probe. cDNA clones HP57 and 3F appear to be derived from the same gene; both clones differ in the 5' and 3' untranslated sequence as well as in the C terminal tail (C-terminal of the kinase domain).

ARK-1

(3F)

Figure 6

④

GAATTCCGGCAGGAGGCTAGCCCAAACTCTGCCCTCATTCCTGCAAGGCTCCTAGACCGAGGACCCCCGG 70
NCTGAGGGTGGGCCCCCATCCAGTCCCQGGAGGCTGCCGCGCCAGCTGCQCCBAGCBAACCCCTCCCCG 140
GCTCAGCCCGGTCCGGGGCCCGCCCGACCCAGCCGCCCTCCAGCCTGGCGGTGCAACTCGCGCCG 210
CGCQGTGGAGGGGAGGTGGCCCCGTCCGCCAAGCTAGCGCCCCGCCACCCGAGAGCQGGCCAGAGG 280
GACCATGACCTTGGGCTCCCCAGGAAAGGCCTTCTGATGCTGCTGATGGCCTTGGTGACCCAGGGAGAG 350
H T L G S P R K G L L H L L H A L V T G G D
CCTGTGAAGCCGTCTCGGGGCCCGCTGGTGACCTGCACGTGTGAGAGCCACATTGCAAGGGGCTACCT 420
P V K P S R G P L Y T C T C E S P H C K G P T
GCCGGCGGGCCTGGTGCACAGTAGTGTGTGCGGGAGGAGGGGAGGCCACCCCAAGAACATCGGGGCTG 480
C R G A W C T Y V L V R E E G R H P O E H R G C
CGGAACTTGACAGGAGCTCTGCAGGGGGCCGCCACCGAGTTGCTCAACCACTACTCTGCGACAGC 560
G N L H R E L C R O R P T E F V N H Y C C O S
CAGCTGTGCAACCAACGTGTCCCTGGTCTGGAAGGCCACCAACCTCTTCGGAGCAGCCGGGAACAG 630
H L C N H N V S L V L E A T O P P S E O P G T
ATGCCAGCTGGCCCTGATCCTGGGCCCCGTGCTGGCCTTGGTGGCCCTGGTGGCCCTGGTGTCTGGG 700
D G O L A L I L G P Y L A L L A L V A L G V L G
CCTGTGGCATGTCCGACGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG 770
L W H V R R R O E K O R G L H S E L G S S L
ATCCTGAAAGCATCTGAGCAGGGCGACAGGATGTTGGGGGACCTCCTGGACAGTGAAGTGCACACAGGA 840
I L K A S E O G D T H L G D L L D S D C T T G
CTGGCTCAGGGCTCCCTTCTGCTGTCAGAGGACAGTGGCAGGCGAGGTTGCCCTTGGTGGAGTGTGGG 910
S G S G L P F L V O R T V A R O V A L V E C V G
AAAAGGCCCTATGGCGAAGTGTGCGGGGCTTCTGCGACGCTGAGAGTGTGGCCGTCAAGATCTTCTCC 980
K G R Y G E V W R O L W H O E S V A V K I F S
TCGAGGGATGAACAGTCTGTTCCGGGAGAGTGAAGATCTATAACACAGTATTGCTCAGACACGACAACA 1050
S R D E O S W F R E T E I Y N T V L L R H D N
TCCTAGGCTTCATCGCCTCAGACATGACCTCCCGCAACTCGAGCACGAGCTGTGGCTCATCAGGACTA 1120
I L G F I A S D H T S R N S S T O L V L I T H Y
CGACGAGCAGGCTCCCTCTACGACTTCTGCAAGACACAGGCTGGAAGCCCATCTGGCTGTGAGGCTA 1180
H E H G S L Y D F L O R O T L E P H L A L R L
CCTGTGTCGGCGGATGCGGCTGCGCGACCTGCACGTGGAGATCTTCGGTACACAGGGCAAAACAGCCA 1260
A V S A A C G L A H L H V E I F G T O G K P A
TTGCCACCGGCACTTCAAGAGCCGCAATGTGTGTGTCAGAGCAACCTGCAGTGTTCATCGCCGACCT 1330
I A H R D F K S R N V L V K S N L Q C C I A D L
GGGCGTGGCTGTGATGCATCACAGGCGAGGATTACCTGGACATCGGCAACAACCCGAGAGTGGGACCC 1400
G L A V H H S O G S D Y L D I G N N P R Y B T
AAGCGTACATGGCAGCCGAGGTGCTGGACGAGCAGATCCGCACGAGTCTTGTAGTCTTACAAGTGA 1470
K R Y H A P E V L D E O I R T D C F E S Y K V
CTCAGATCTGGGCTTTGGCCTGCTGTGGGAGATTGCCCGCGAGCATGCTGAATGGCATCGTGA 1540
T D I W A F G L V L V E I A R R T I V N S I V E

(3F)

⑤

GGACTATAGACCACCCTTCTATGATGTGGTGCCCAATGACCCAGCTTTGAGGACATGAAGAAGGTGGTG 1610
 D Y R P P F Y D V V P N D P S F E D H K K V V
 TGTGTGGATCAGCAGACCCCAACCATCCCTAACCGGCTGGCTGCAGACCCGGTCCCTCTCAGGCCTAGCTC 1680
 C V D Q Q T P T I P N R L A A D P V L S G L A
 AGATGATCGGGCAGTGTCTGCTACCCAAACCCCTCTGCCCGACTCACCGCGCTCGGGATCAAGAAGACACT 1750
 Q H H R E C W Y P N P S A R L T A L R I K K T L
 ACAAAAAAATTAGCAACAOTCCAGAGAAGCCTAAAGTGATTCAATAGCCCAAGGACACCTGATTCCTTTC 1820
 Q K N .
 TGCCTGCAAGGGGGCTGGGGGGGTGGGGGGCAGTGGATGGTGCCCTATCTCGGTAGAGGTAGTGTGAGTGT 1890
 GGTGTGTGCTGGGGATGGGCAGCTGCGCCTGCTGCTCGGGCCCGACGCCACCCAGCCAAATAACAGCT 1960
 GGGCTGAAACCTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT 2003

Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 1 (ARK-1). The complete sequence of cDNA clone 3F is shown, which was obtained by screening a human foreskin fibroblast cDNA library with PCR product 11.3 as a probe. cDNA clones 3F and HP57 appear to be derived from the same gene; both clones differ in the 5' and 3' untranslated sequence as well as in the C terminal tail (C-terminal of the kinase domain).

CTCCGAGTACCCAGTGACCAAGAGTGAAGAGAGCTCTGAACGAGGGCACGCGGCTTGAAGCACTGTGGGC 70
AGATGTGACCAAGAGCCTGCATTAAGTTGTACAATGGTAGATGGAGTGATGATTCTTCTGTGCTTATCA 140
TGATTGCTCTCCCCTCCCCTAGTATGGAAGATGAGAAGCCCAAGGTCAACCCCAAACCTCTACATGTGTGT
H V D G V H I L P V L I 210
M I A L P S P S H E D E K P K V N P K L Y H C V
GTGTGAAGGTCTCTCTGCGGTAATGAGGACCACTGTGAAGGCCAGCAGTGCTTTTCTCACTGAGCATC 280
C E G L S C G N E D H C E G Q Q C F S S L S I
AACGATGGCTTCCACGTCTACCAGAAAGGCTGCTTCCAGGTTTATGAGCAGGGAAGATGACCTGTAAGA 350
N O G F H V Y Q K G C F Q Y Y E Q G K M T C K
CCCCGCGCTCCCTGGCCAAGCTGTGGAGTGCTGCCAAGGGGACTGGTGTAAACAGGAACATCACGGCCCA 420
T P P S P G O A V E C C Q G D W C N R N I T A Q
GCTGCCCACTAAAGGAAAATCCTTCCCTGGAACACAGAATTTCCACTTGGAGGTTGGCCTCATTATTCTC 490
L P T K G K S F P G T O N F H L E V G L I I L
TCTGTAGTGTTYGCAATATGTCTTTAGCCTGCCTGCTGGGAGTTGCTCTCCGAAAATTTAAAAGGCGCA 560
S V V F A V C L L A C L L G V A L R K F K R R
ACCAAGAACGCTCAATCCCCGAGACGTGGAGTATGGCACTATCGAAGGQCTCATCACCACCAATGTTGG 630
N O E R L N P R D V E Y G T I E G L I T T N V G
AGACAGCACTTTAGCAGATTTATTGGATCATTGCTGTACATCAGGAAGTGGCTCTGGTCTTCTTTTCTG 700
D S T L A D L L D H S C T S G S G S G L P F L
GTACAAAGAACAGTGGCTCGCCAGATTACACTGTTGGAGTGTGTGCGGAAAGGCAGGTATGGTGAGGTGT 770
V O R T V A R O I T L L E C V G K G R Y G E V
GGAGGGGCAGCTGGCAAGGGGAAAATGTTGCCGTGAAGATCTTCTCTCCCGTGATGAGAAGTCATGGTT 840
W R G S W Q G E N V A V K I F S S R D E K S W F
CAGGGAACGGAATTGTACAACACTGTGATGCTGAGGCATGAAAATATCTTAGGTTTCATTGCTTCAGAC 910
R E T E L Y N T V M L R H E N I L G F I A S D
ATGACATCAAGACACTCCAGTACCCAGCTGTGGTTAATTACACATTATCATGAAATGGGATCGTTGTACG 980
M T S R H S S T O L W L I T H Y H E M G S L Y
ACTATCTTCAGCTTACTACTCTGGATACAGTTAGCTGCCCTTGAATAGTGCTGTCCATAGCTAGTGGTCT 1050
D Y L O L T T L D T V S C L R I V L S I A S G L
TGCACATTTGCACATAGAGATATTTGGGACCCAAGGGAACCCAGCCATTGCCCATCGAGATTTAAAGAGC 1120
A H L H I E I F G T O G K P A I A H R D L K S
AAAAATATTCTGGTTAAGAAGAATGGACAGTGTTCATAGCAGATTTGGGCTGGCAGTCATGCATTCCC 1190
K N I L V K K N G O C C I A D L Q L A V M H S
AGAGCACCAATCAGCTTGATGTGGGGAACAATCCCGTGTTGGGCACCAAGCGCTACATGGCCCCCAAGT 1260
Q S T N O L D V G N N P R V G T K R Y H A P E V
TCTAGATGAAACCATCCAGGTGGATTGTTTCGATTCTTATAAAAGGGTCGATATTTGGGCCTTTGCACTT 1330
L D E T I O V D C F D S Y K R V D I W A F G L
GTTTTCTGGGAAGTGCCAGCGGATGCTGAGCAATGGTATAGTGGAGGATTACAAGCCACCGTTCTACG 1400
V L W E V A R R H V S N G I V E D Y K P P F Y
ATGTGGTCCCAATGACCAAGTTTTGAAGATATCAGGAAGGTAGTCTGTGTGGATCAACAAGGCCAAA 1470
D V V P N D P S F E D M R K V V C V D Q Q R P N

CATACCCAACAGATGGTTCTCAGACCCGACATTAACCTCTCTGGCCAAGCTAATGAAAGAATGCTGGTAT 1640
 I P N R W F S D P T L T S L A K L M K E C W Y
 CAAAATCCATCCGCAAGACTCAGGCACTGCGTATCAAAAAGACTTTGACCAAAATTGATAATTCCTCG 1610
 Q N P S A R L T A L R I K K T L T K I D N S L
 ACAAATTGAAAAGTACTGTTGACATTTTCATAGTGTCAAGAAGGAAGATTTGACGTTGTTGTCATTGTC 1680
 D K L K T D C
 CAGCTGGGACCTAATGCTGGCTGACTGGTTGTGAGAATGGAATCCATCTGTCTCCCTCCCAAATGGCT 1760
 GCTTTGACAAGGCAGACGTCGTACCCAGCCATGTGTTGGGGAGACATCAAACCACCCTAACCTCGCTCG 1820
 ATGACTGTGAACTGGGCATTTACGAAGTGTTCACACTGCAGAGACTAATGTTGGACAGACACTGTTGCA 1890
 AAGGTAGGGACTGGAGGAACACAGAGAAATCCTAAAAGGATCTGGGCATTAAGTCAGTGGCTTTGCATAG 1960
 CTTTCACAGTCTCCTAGACACTCCCCACGGGAAACTCAAGGAGGTGGTGAATTTTTAATCAGCAATATTG 2030
 CCTGTGCTTCTCTTCTTTATTGCACTAGGAATTCTTTGCATTCTTACTTGCCTGTTACCTTAATTTTA 2100
 AAGACCCAACCTTGCCAAAATGTTGGCTGCGTACTCCACTGGTCTGTCTTTGGATAATAGGAATTCAATT 2170
 GGCAAAACAAAATGTAATGTCAGACTTTGCTGCATTTTACACATGTGCTGATGTTTACAATGATGCCGAA 2240
 CATTAGGAATTGTTTATACACAACCTTTGCAAATTATTTATTACTTGTGCACTTAGTAGTTTTTACAAAAC 2310
 TGCTTTGTGCATATGTTAAACCTTATTTTTATGTGCTTATGATTTTATTACAGAAATGTTTTTAACAC 2380
 TATACTCTAAAATGGACATTTTCTTTTATTATCAGTTAAAATCAGTTTTAAGTGCTTCACATTTGTATG 2450
 TGTGTAGACTGTAACTTTTTTTCAGTTCATATGCAGAACGTATTTAGCCATTACCCACGTGACACCACCG 2520
 AATATATTATCGATTTAGAAAGCAAAGATTTTCACTAGAAATTTAGTCTGAACGCTACGGGGAAAAATGCA 2590
 TTTTCTTCAGAAATTATCCATTACGTGCATTTAACTCTGCCAGAAAAAATAACTATTTTGTTTTAATCT 2660
 ACTTTTGTATTTAGTAGTTATTTGTATAAATTAATAAACTGTTTTCAAAAAAAA → 2717

Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 2 (ARK-2). The complete sequence of cDNA clone HP 53 is shown, which was obtained by screening a human placenta cDNA library with PCR product 11.2 as a probe.

(4)

GAATTCGGTTGCTGCTCGGCTCCGCGCCGAGGGCTGGAGGATGCGTTCCCTGGGGTCCGGACTTATGAAAA 70
TATGCATCAGTTTAATACTGTCTTGAATTCATGAGATGGAAGCATAGGTCAAAGCTGTTTGGAGAAAAAT 140
CAGAAGTACAGTTTTATCTAGCCACATCTTGGAGGAGTCGTAAGAAAGCAGTGGGAGTTGAAGTCATTGT 210
CAAGTGCTTGGCATCTTTTACAAGAAAATCTCACTGAATGATAGTCATTAAATTGGTGAAGTAGCAAGA 280
CCAATTATTAAGGTGACAGTACACAGGAACATTACAATTGAACAATGACTCAGCTATACATTTACATC 350
AGATTATTGGGAGCCTATTTGTTTCATCATTCTCGTGTTCAGGACAGAATCTGGATAGTATGCTTCATG M T O L Y I Y I 420
R L L G A Y L F I I S R V Q G O N L D S M L H
GCCTGGGATGAAATCAGACTCCGACCAGAAAAAGTCAGAAAATGGAGTAACCTTAGCACCAGAGGATAC 480
G T G M K S D S D Q K K S E N G V T L A P E D T
CTTGCTTTTTTAAAGTGCTATTGCTCAGGGCACTGTCAGATGATGCTATTAATAACACATGCATACT 560
L P F L K C Y C S G H C P D D A I N N T C I T
AATGGACATTGCTTTGCCATCATAGAAGAAGATGACCAGGGAGAAACCACATTAGCTTCAGGGTGTATGA 630
N G H C F A I I E E D D O G E T T L A S G C H
AATATGAAGGATCTGATTTTCACTGCAAGATTCTCCAAAAGCCAGCTACGCCGACAATAGAATGTTG 700
K Y E G S D F O C K D S P K A O L R R T I E C C
TCGGACCAATTTATGTAACCAGTATTGCAACCCACACTGCCCCCTGTTGTCATAGGTCCGTTTTTTGAT 770
R T N L C N O Y L O P T L P P V V I G P F F D
GGCAGCATTGATGGCTGGTTTTGCTCATTCTATGGCTGTCTGCATAATTGCTATGATCATCTTCTCCA 840
G S I R W L V L L I S H A V C I I A M I I F S
GCTGCTTTTGTACAAACATTATTGCAAGAGCATCTCAAGCAGACGTCGTTACAATCGTGATTGGAACA 910
S C F C Y K H Y C K S I S S R R R Y N R D L E O
GGATGAAGCATTATTTCCAGTTGGAGAATCACTAAAAGACCTTATTGACCAGTCACAAAGTTCTGGTAGT 980
D E A F I P V G E S L K D L I D O S O S S G S
GGGTCTGGACTACCTTTATTGGTTTCAGCGAACTATTGCCAAACAGATTGAGATGGTCCGGCAAGTTGCTA 1050
G S G L P L L V Q R T F A K Q I Q M V R O V G
AAGGCCGATATGGAGAAGTATGGATGGCAAAATGGCGTGCGGAAAAAGTGGCGGTGAAAGTATTCTTTAC 1120
K G R Y G E V W H G K W R G E K V A V K V F F T
CACTGAAGAAGCCAGCTGTTTCGAGAAACAGAAATCTACCAAAGTGTGCTAATGGCCCATGAAAACATA 1190
T E E A S W F R E T E I Y Q T V L M R H E N I
CTTGGTTTCATAGCGGCAGACATTAAAGGTACAGGTTCTGGACTCAGCTCTATTGATTACTGATTACC 1260
L G F I A A D I K G T G S W T O L Y L I T O Y
ATGAAAATGGATCTCTATGACTTCTGAAATGTGCTACACTGGACACCAGAGCCCTGCTTAAATTGGC 1330
H E N G S L Y D F L K C A T L D T R A L L K L A
TTATTAGCTGCCTGTGGTCTGTGCCACCTGCACACAGAAATTTATGGCACCAAGGAAAGCCCGCAATT 1400
Y S A A C G L C H L H T E I Y G T O G K P A I
GCTCATCGAGACCTAAAGAGCAAAAACATCCTCATCAAGAAAAATGGGAGTTGCTGCATTGCTGACCTGG 1470
A H R D L K S K N I L I K K N G S C C I A D L
GCCTTGCTGTAAATTCAACAGTGACACAAATGAAGTTGATGTGCCCTTGAATACCAGGGTGGCCACCAA 1540
G L A V K F N S D T N E V D V P L N T R V C T K

ACGCTACATGGCTCCCGAAGTGCTGGACGAAAGCCTGAACAAAAACCACTTCCAGCCCTACATCATGGCT 1810
 R Y M A P E Y L D E S L N K N H F Q P Y I M A
 GACATCTACAGCTTCGGCCTAATCATTTGGGAGATGGCTCGTCTGTATCACAGGACGGATCGTGGAAG 1880
 D I Y S F G L I I W E M A R R C I T G G I V E
 AATACCAATTGCCATATTACAACATGGTACCGAGTGATCCGTCATACGAAGATATGCGTGAGGTTGTGTG 1750
 E Y Q L P Y Y N H V P S D P S Y E D H R E V V C
 TGTCAAACGTTTGGCGCAATTGTGTCTAATCGGTGGAACAGTGATGAATGTCTACGAGCAGTTTGAAG 1820
 V K R L R P I V S N R W N S D E C L R A V L K
 CTAATGTCAGAATGCTGGGCCCAATCCAGCCTCCAGACTCACAGCATTGAGAATTAAGAAGACGCTTG 1890
 L M S E C W A H N P A S R L T A L R I K K T L
 CCAAGATGGTTGAATCCCAAGATGTAAAAATCTGATGGTTAAACCATCGGAGGAGAAACTCTAGACTGCA 1960
 A K M V E S Q D V K I
 AGAACTGTTTTTACCCATGGCATGGGTGGAATTAGAGTGGAATAAGGATGTTAACTTGGTTCTCAGACTC 2030
 TTTCTTCACTACGTGTTACAGGCTGCTAATATTAAACCTTTCAGTACTCTTATTAGGATACAAGCTGGG 2100
 AACTTCTAAACACTTCATTCTTTATATATGGACAGCTTTATTTTAAATGTGGTTTTTGTATGCCTTTTTT 2170
 AAGTGGGTTTTTATGAAGTGCATCAAGACTTCAATCCTGATTAGTGTCTCCAGTCAAGCTCTGGGTACTG 2240
 AATTGCCTGTTTCATAAAACGGTGCTTTCTGTGAAAGCCTTAAGAAGATAAATGAGCCGAGCAGAGATGGA 2310
 GAAATAGACTTTGCCTTTTACCTGAGACATTCAAGTTCGTTTGTATTCTACCTTTGTAAAACAGCCTATAG 2380
 ATGATCATGTGTTTGGGATACTGCTTATTTTATCATAGTTTGTCTGTCTCCTTAGTGATGTGTGTGTGT 2450
 CTCCATGCACATGCACGCCGGGATTCTCTGCTGCCATTTGAATTAGAAGAAAATAATTTATATGCATGC 2520
 ACAGGAAGATATTGGTGGCCGGTGGTTTTTGTGCTTTAAAAATGCAATATCTGACCAAGATTCCCAATC 2590
 TCATACAAGCCATTTACTTTGCAAGTGAGATAGCTTCCCCACCAGCTTTATTTTAAACATGAAAGCTGA 2660
 TGCCAAGGCCAAAAGAAGTTTAAAGCATCTGTAAATTTGGACTGTTTTCTTCAACCACCATTTTTTTTG 2730
 TGGTTATTATTTTTGTACGGAAAGCATCCTCTCCAAGTTGGAGCTTCTATTGCCATGAACCATGCTTA 2800
 CAAAGAAAGCACTTCTTATTGAAGTGAATTCCTGCATTTGATAGCAATGTAAGTGCCTATAACCATGTTT 2870
 TATATTCTTTATTCTCAGTAACTTTTAAAGGGAAGTTATTTATATTTTGTGTATAATGTGCTTTATTTG 2940
 CAAATCACCCCGACAGCAACGAATTC 2968

Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 3 (ARK-3). The sequence of cDNA clone ONF5 is shown, which was obtained by screening a human foreskin fibroblast cDNA library with the kinase domain of cDNA clone ON11 as a probe. (cDNA clone ON11 was obtained from human fibroblast cDNA library using oligonucleotide derived from PCR product 5.1 as a probe)

ARX-4

Figure 1

⑧

TTGCTGCTGACAATAAAGATAATGGCACCTGGACACAGCTGTGGCTTGTTTCTGACTATCATGAGCACG 70
I A A D N K D N G T W T Q L W L V S D Y H E H
GGTCCCTGTTTGNTTATCTGAACCGGTACACAGTGACAATTGAGGGGATGATTAAGCTGGCCTTGCTCTGC 140
G S L F ? Y L N R Y T V T I E G M I K L A L S A
TGCTAGTGGGCTGGCACACCTGCACATGGAGATCGTGGGCACCCAAGGGAAGCCTGGAATTGCTCATCGA 210
A S G L A H L H M E I V G T Q G K P G I A H R
GACTTAAAGTCAAAGAACATTCTGGTGAAGAAAAATGGCATGTGTGCCATAGCAGACCTGGGCCTGGCTG 280
D L K S K N I L V K K N G M C A I A D L G L A
TCCGTCATGATGCAGTCACTCACACAATTGACATTGCCCCGA 322
V R H D A V T H T I D I A P

Partial nucleotide sequence and deduced amino acid sequence of a PCR product 11.1. This PCR product was obtained using human erythroid leukemia cells as mRNA source in the cDNA preparation and primers B3-S and E8-AS in the PCR.

GGCGAGGCGAGGTTTGTCTGGGGTGAGGCACGGCGCGGGCCGGGCCGGGCCACAGGCGGTGGCGGC 70
GGGACCATGGAGGCGGGCGGTGCTGCTCCGCGTCCCCGGCTGCTCCTCCTCGTGTGGCGGCGGGCGGC 140
H E A A V A A P R P R L L L L V L A A A A
CGGCGGCGGGCGGCGCTGCTCCCGGGGGCGACGGCGTTACAGTGTCTTCTGCCACCTCTGTACAAAAGACAA 210
A A A A A L L P G A T A L Q C F C H L C T K D N
TTTTACTGTGTGACAGATGGGCTCTGCTTTGTCTCTGTACAGAGACCACAGACAAAGTTATACACAAC 280
F T C V T D G L C F V S V T E T T D K V I H N
AGCATGTGTATAGCTGAAATTGACTTAATTCCTCGAGATAGGCCGTTTGTATGTGCACCCTCTTCAAAAA 350
S M C I A E I D L I P R D R P F V C A P S S K
CTGGGTCTGTGACTACAACATATTGCTGCAATCAGGACCATTTGCAATAAAATAGAACTTCCAACACTGT 420
T G S V T T T Y C C N Q D H C N K I E L P T T V
AAAGTCATCACCTGGCCTTGGTCTGTGGAAGTGGCAGCTGTCATTGCTGGACCAGTGTCTTCTGTCTGC 490
K S S P G L G P V E L A A V I A G P V C F V C
ATCTCACTCATGTTGATGGTCTATATCTGCCACAACCGCACTGTCAATCACCATCGAGTGCCAAATGAAG 560
I S L M L H V Y I C H N R T V I H H R V P N E
AGGACCCCTTCATTAGATCGCCCTTTTATTTTCAGAGGGTACTACGTTGAAAGACTTAATTTATGATATGAC 630
E D P S L D R P F I S E G T T L K D L I Y D M T
AACGTCAGGTTCTGGCTCAGGTTTACCATTGCTTGTTCAGAGAACAATTGCGAGAAGTATTGTGTTACAA 700
T S G S G S G L P L L V Q R T I A R T I V L Q
GAAAGCATTGGCAAAGGTCGATTGGGAGAAGTTGGAGAGGAAAGTGGCGGGGACAAGAAGTTCTGTGA 770
E S I G K G R F G E V W R G K W R G E E V A V
AGATATTCTCCTCTAGAGAAGAAGCTTCGTGGTTCCGTGAGGCAGAGATTTATCAAAGTGAATGTTACG 840
K I F S S R E E R S W F R E A E I Y Q T V M L R
TCATGAAAACATCCTGGGATTTATAGCAGCAGACAATAAGACAATGGTACTTGGACTCAGCTCTGGTTC 910
H E N I L G F I A A D N K D N G T W T Q L W L
GTGTCAGATTATCATGAGCATGGATCCCTTTTTGATTACTTAAACAGATACACAGTTACTGTGGAAGCAA 980
V S D Y H E H G S L F D Y L N R Y T V T V E G
TGATAAAACTTGCTCTGTCCAGCGCGAGCGGTCTTGCCCATCTTCACATGGAGATTGTTGGTACCCAAGC 1050
M I K L A L S T A S G L A H L H M E I V G T Q G
AAAGCCAGCCATTGCTCATAGAGATTTGAAATCAAAGAATATCTTGGTAAAGAAGAATGGAAGTCTGT 1120
K P A I A H R D L K S K N I L V K K N G T C C
ATTGCAGACTTAGGACTGGCAGTAAGACATGATTACGCCAGAGATACCATTGATATTGCTCCAAACCACA 1180
I A D L G L A V R H D S A T D T I D I A P N H
GAGTGGGAACAAAAAGGTACATGGCCCCCTGAAGTTCTCGATGATTCCATAAATATGAAACATTTGAATC 1260
R V G T K R Y H A P E V L D D S I N H K H F E S
CTTCAAACGTGCTGACATCTATGCAATGGGCTTAGTATTCTGGGAAATTGCTCGACGATGTTCCATTGGT 1330
F K R A D I Y A H G L V F W E I A R R C S I G
GGAATTCATGAAGATTACCAACTGCCTTATTATGATCTTGTACCTTCTGACCCATCAGTTGAAGAAATGA 1400
G I H E D Y Q L P Y Y D L V P S D P S V E E H
GAAAAGTTGTTGTGAACAGAAGTTAAGGCCAAATATCCCAAACAGATGGCAGAGCTGTGAAGCCTTGAG 1470
R K V V C E Q K L R P N I P N R W Q S C E A L R

(7)

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AGTAATGGCTAAAATTATGAGAGAATGTTGGTATGCCAATGGAGCAGCTAGGCTTACAQCATTGCBGATT 1540
  V H A K I H R E C W Y A N G A A R L T A L R I
AAGAAACATTATCGCAACTCAGTCAACAGGAAGGCATCAAAATGTAATTCTACAGCTTTGCCTGAACTC 1610
  K K T L S Q L S Q Q E G I K H .
TCCTTTTTCTTCAGATCTGCTCCTGGGTTTTAATTTGGCAGGTCAGTTGTTCTACCTCACTGAGAGGGA 1680
ACAGAAGGATATTGCTTCCTTTTGCAGCAGTGTAAATAAAGTCAATTAAAACTTCCCAGGATTTCTTTGG 1750
ACCCAGGAAACAGCCATGTGGGTCCTTTCTGTGCACTATGAACGCTTCTTTCCCAGGACAGAAAATGTGT 1820
AGTCTACCTTTATTTTTTATTAACAAAACCTGTTTTTTAAAAAGATGATTGCTGGTCTTAACCTTAGGTA 1890
ACTCTGCTGTGCTGGAGATCATCTTTAAGGGCAAAGGAGTTGGATTGCTGAATTACAATGAAACATGTCT 1960
TATTACTAAAGAAAGTGATTTACTCCTGGTTAGTACATTCTCAGAGGATTCTGAACCACTAGAGTTTCCT 2030
TGATTCAGACTTTGAATGTACTGTTCTATAGTTTTTCAGGATCTTAAACTAACACTTATAAACTCTTA 2100
TCTTGAGTCTAAAAATGACCTCATATAGTAGTGAGGAACATAATTCATGCAATTGTATTTGTATACTAT 2170
TATTGTTCTTTCACTTATTCAGAACATTACATGCCTTCAAAATGGGATTGTACTATACCAGTAAGTCCA 2240
CTTCTGTGTCTTTCTAATGGAAATGAGTAGAATTGCTGAAAGTCTCTATGTTAAACCTATAQTGTTT 2308

```

Nucleotide sequence and deduced amino acid sequence of activin receptor related kinase - 5 (ARK-5). The sequence of cDNA clone EMBLA is shown, which was obtained by screening a human HEL cDNA library with PCR products 11.1 and 11.2 as a probe.

Figure 11

COMPARISON OF KINASE DOMAINS

percent similarity

	ARK-1	ARK-2	ARK-3	11.1 PCR PR/ARK-4	ARK-5/KD	TGFBRII/KD	ActR-II/KD	ActR-IIB/KD	dat-1/KD	
1										
2	70.5									1
3	52.1	45.1								2
4	40.2	59.8	61.7							3
5	55.2	65.5	65.5	91.6						4
6	34.5	35.1	33.8	35.5	39.3					5
7	32.6	37.9	38.3	38.4	37.8	42.2				6
8	32.2	34.4	33.7	34.6	35.5	41.6	78.0			7
9	32.6	36.2	35.2	45.8	39.3	29.9	32.9	27.1		8
									9	9

ARK-1

ARK-2

ARK-3

11.1 PCR PR/ARK-4

ARK-5/KD

TGFBRII/KD

ActR-II/KD

ActR-IIB/KD

dat-1/KD

dendrogram

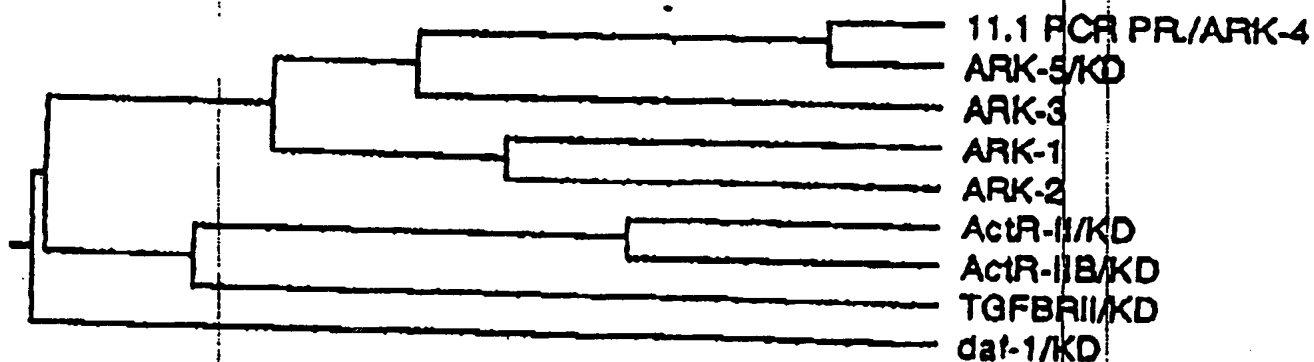


Figure 1

Figure 12

9

Nucleotide sequence and deduced amino acid sequence of a PCR product 5.1. This PCR product was obtained using human erythroid leukemia cells as mRNA source in the cDNA preparation and primers B7-S and E8-AS in the PCR. The primer sequences are included ; these sequences, in particular sequence forming the restriction sites, may not correspond with the gene sequence from which the PCR product was derived.

Nov. 1

3.1

Fig 13

10

GCGGCCACACTCTGCGGCTCCCCCATGTACATGGCTCCCGAATTC 45
A A T L C G S P M Y M A P E F

Partial nucleotide sequence and deduced amino acid sequence of a PCR product 3.1. This PCR product was obtained using human erythroid leukemia cells as mRNA source in the cDNA preparation and primers B7-S and E8-AS in the PCR. The primer sequence is included ; this sequence, in particular the sequence forming the restriction site, may not correspond with the gene sequence from which the PCR product was derived.

None

112-6

Figure 14

(11)

```
GGATCCGCTGACATCAAGACCTAAGAACATCATCGTCAGCAGCCAGGCCACATCAAACCTGACCGACTTT 70
D P L T S R P K N I I V S S Q A H I K L T D F
GGACTCTGCAAGGAGTCTATCCATGAGGGCGCCGTCACACCTTCTGCGGCACCATTGAGTACATGG 140
G L C K E S I H E G A V T H T F C G T I E Y M
CCCCTGAATTC 151
A P E F
```

Nucleotide sequence and deduced amino acid sequence of a PCR product 1/2-6. This PCR product was obtained using human erythroid leukemia cells as mRNA source in the cDNA preparation and primers B7-S and E8-AS in the PCR. The primer sequences are included ; these sequences, in particular sequence forming the restriction sites, may not correspond with the gene sequence from which the PCR product was derived.

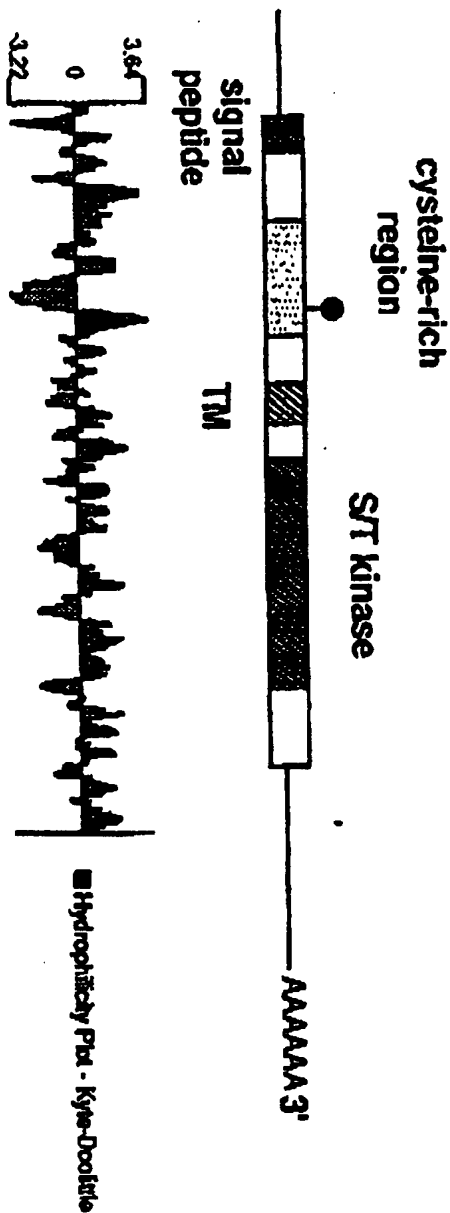
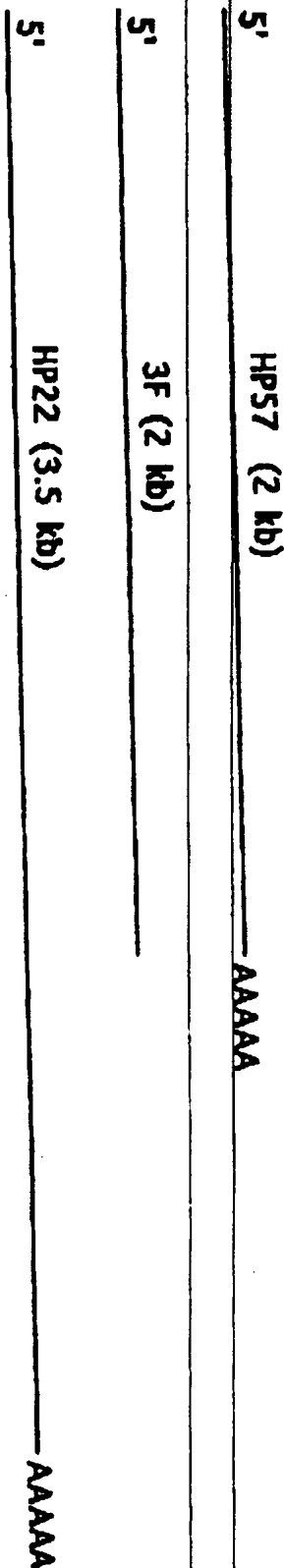
(12)

GGATCCCGGACATTAAATGCAAGAACATTCTGGTGAAGAAAAATGGCATGTGTGCCATAGCAGACCTGGG 70
 R I P D I K C K N I L V K K N G M C A I A D L G
 CCTGGCTGTCCGTCATGATGCAGTCACTGACACCATTGACATTGCCCGAATCAGAGGGTGGGGACCAA 140
 L A V R H D A V T D T I D I A P N Q R V G T K
 CGATACATGGCCCCAGAATTC 161
 R Y M A P E F

Nucleotide sequence and deduced amino acid sequence of a PCR product 1/2-7.
 This PCR product was obtained using human erythroid leukemia cells as mRNA
 source in the cDNA preparation and primers B7-S and E8-AS in the PCR. The primer
 sequences are included ; these sequences, in particular sequence forming the
 restriction sites, may not correspond with the gene sequence from which the PCR
 product was derived.

Figure 1

ARK-1



EXPRESSION : RESTRICTED
HUMAN FIBROBLASTS
5 AND 1.8 KB

F0217

ARK-2

5'

HP64 (2.5 kb)

5'

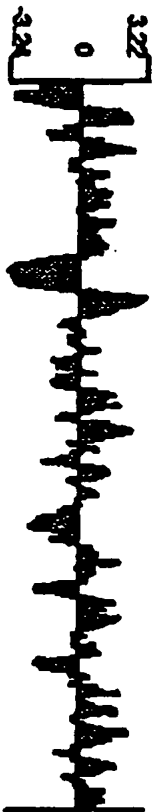
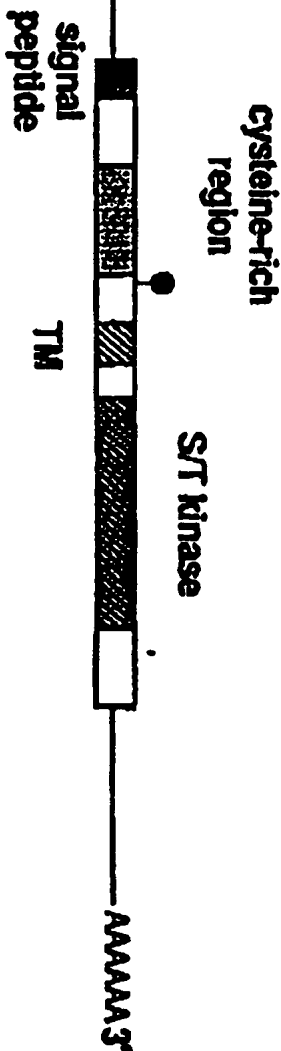
HP 53 (3 kb)

5'

HP64 (4 kb)

AAAAA

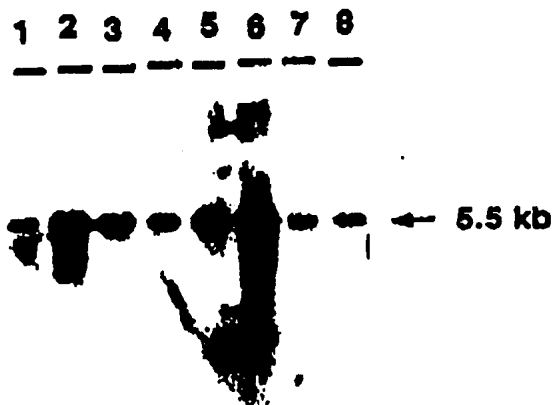
AAAAA



Hydropathy Plot - Kyte-Doolittle

EXPRESSION : UBQUITOUS
8 AND 4 kb

Figure 22

**Northern blot analysis**

mRNA (2 microgram) from different human tissues (Clontech, CA, USA) was hybridized with a radiolabeled 1 kb EcoRI fragment of cDNA clone EMBLA, corresponding to ARK-5, in the presence of 50 % formamide and 5 x SSC. Filter was washed with 0.5 x SSC, 0.1 % SDS at 55 C. Lane 1, heart; lane 2, brain; lane 3, placenta; lane 4, lung; lane 5, liver; lane 6, skeletal muscle; lane 7, kidney; lane 8, pancreas.